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Abstract:	<p>Introduction: Corn snakes are a very common pet reptile species, yet there is an absence of evidence-based literature standardising collection of electrocardiographs (ECG) or detailing ECG deflection morphology in the normal animal. We describe a well-tolerated, reproducible technique and detail the cardiac cycle in terms of lead 2 equivalent waveforms and intervals.</p> <p>Animals: 29 adult corn snakes.</p> <p>Materials and methods: This prospective study evaluated under species-appropriate, standardised conditions, a technique for producing standard six-lead ECG tracings. Lead 2 equivalent cardiac cycles were described in detail and statistically analysed for gender, weight, length, heart rate and mean electrical axis.</p> <p>Results: High-quality tracings demonstrated common ECG characteristics for this species, including: no Q, S or SV waves, prolonged PR and RT intervals, rhythmic oscillation of the baseline, short TP segments and a right displaced mean electrical axis. An influence of gender, weight or length on heart rate and mean electrical axis was not identified.</p> <p>Conclusions: To the authors' knowledge, this is the first study to describe a standardised technique for recording ECG in significant numbers of normal corn snakes. Ranges have been provided that may be of diagnostic value or form the basis for future development of reference intervals for this species</p>

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Standardization of electrocardiographic examination in corn snakes (*Pantherophis guttatus*)

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Abstract

Introduction: Corn snakes are a very common pet reptile species, yet there is an absence of evidence-based literature standardising collection of electrocardiographs (ECG) or detailing ECG deflection morphology in the normal animal. We describe a well-tolerated, reproducible technique and detail the cardiac cycle in terms of lead 2 equivalent waveforms and intervals.

Animals: 29 adult corn snakes.

Materials and methods: This prospective study evaluated under species-appropriate, standardised conditions, a technique for producing standard six-lead ECG tracings. Lead 2 equivalent cardiac cycles were described in detail and statistically analysed for gender, weight, length, heart rate and mean electrical axis.

Results: High-quality tracings demonstrated common ECG characteristics for this species, including: no Q, S or SV waves, prolonged PR and RT intervals, rhythmic oscillation of the baseline, short TP segments and a right displaced mean electrical axis. An influence of gender, weight or length on heart rate and mean electrical axis was not identified.

Conclusions: To the authors' knowledge, this is the first study to describe a standardised technique for recording ECG in significant numbers of normal corn snakes. Ranges have been provided that may be of diagnostic value or form the basis for future development of reference intervals for this species.

Introduction

Evaluation of cardiac performance and diagnosis of cardiac disease in the ophidian patient has historically received little attention, even in captive reptile species, such as the corn snake that are common both in private and zoological collections.

For captive reptiles poor husbandry is ubiquitous and pervasive, and could predispose to cardiac disease either through immunocompromise or nutritional/metabolic derangement.^{1 2} Indeed, both primary and secondary forms of cardiac disease are widely described in literature and demonstrate the broad spectrum of conditions from which snakes may suffer.³⁻¹² Despite this, the prevalence of cardiac disease in snakes is unknown, and our understanding of the impact of cardiac disease on morbidity and mortality in even very common pet species, such as corn snakes, is rudimentary in comparison to more familiar companion mammals.

Identification of cardiac disease by both owner and clinician can be very challenging. The typically sedentary lifestyles and highly restricted captive environments of many pet snakes places little demand on the cardiovascular system. A low basal metabolic rate and high tolerance for anaerobic respiration means that heart disease is usually very far advanced before it becomes clinically apparent.^{1 13} Basic cardiac assessment, such as auscultation, has limited value due to poor contact between stethoscope and scales. While more advanced investigations, such as radiography, electrocardiography (ECG) and echocardiography, suffer from a paucity of species-specific reference ranges and standardised methodologies.^{1 13 14}

In common with other non-crocodilian reptiles, the ophidian heart is a three-chambered structure composed of two atria and a single, common ventricle. A reptile-specific pre-filling chamber, the sinus venosus, is situated caudal to the right

atrium¹⁵ and contains spontaneously depolarising pacemaker cells under vagosympathetic control.^{2 16 17} There is no sino-atrial node, atrial-ventricular node or His/Purkinje network that form the specialised electrical conduction system of higher vertebrates, and no annulus fibrosis exists to provide an insulating plane between the atria and ventricle.^{14 15} Instead a canal or “funnel” of atrioventricular myocardium, which resembles the ill-defined AV node region of embryonic mammals and birds, serves as a conduit for electrical conduction into the ventricle. Despite these structural differences, the reptilian ECG waveform is morphologically similar to the mammalian, with a P wave, QRS complex and T wave representing sequential atrial depolarisation, ventricular depolarisation and ventricular repolarisation respectively.¹

13 14

Electrocardiography is widely used in traditional companion mammal species to aid evaluation of cardiac health and to monitor cardiac rhythm while under general anaesthesia, and should also represent an attractive option for common captive snakes to both specialists and general practitioners. Unfortunately, very little literature exists detailing a standardised approach to recording ECGs in snakes or describing wave amplitudes or segment durations. Previous research has typically involved a variety of different study designs, very small study cohorts or mixed species collections and data sets cannot be extrapolated from companion mammals or even other reptilian species.¹⁸⁻²² We sought to correct this deficiency in a common pet species, the corn snake, and provide information useful to cardiac assessment.

We hypothesised that ECGs can be easily recorded from conscious corn snakes in a non-invasive and well tolerated way, and that they take on a recognisable sequence of PQRST deflections amenable to measurement. To

investigate this hypothesis a standardised technique for recording and measuring ECGs was employed.

Materials and Methods

The study population comprised 29 adult corn snakes (*Pantherophis guttatus*) (19 males and 10 females) from privately-owned sources or a locally-based charity rehoming centre, fed whole prey items on a seven-day cycle. As specific ages were unknown snakes were classified as adult based on size (body weight and snout to vent length). Gender was determined by use of a metal probe passed into the hemipenis or musk sac (female if probe depth <5 scutes, male if > 6 scutes). Owners were asked to withhold food for at least four days prior to hospitalisation.^{23 24} None of the snakes were undergoing ecdysis at the time of ECG recording. Ethical approval was obtained from an independent Edinburgh University Veterinary Ethical Review Committee (VERC).

Subjects were deemed healthy based on clinical history and a physical exam which comprised evaluation of demeanour, body condition, respiratory rate and effort, coelomic palpation, and integumentary, oral and cloacal assessments. To allow for thermal acclimation and minimise the influence of travel stress, subjects were housed individually in reptile vivaria for at least four hours prior to ECG examination. Ambient vivarium temperature gradients ranged from 26 - 30°C, which was considered to be within the preferred optimum temperature zone for this species.²⁵

The ECGs were performed at a room temperature of 22-24°C as soon as the snakes were removed from the vivaria. Subjects were supported in ventral recumbency by a single assistant without the use of chemical restraint. When

physical restraint was required, it was limited to gentle manual restriction of movement until the subject settled (figure 1). The heart was estimated at around 25% snout-to-vent length then either identified visually, with ultrasound confirmation, or found directly with the probe and coupling gel (figure 1). Non-traumatic adhesive ECG pads (Ambu ® BlueSensor N) were applied to the lateral skin surface in a four-lead system, 1cm cranial and 1cm caudal to the heart (Figures 1 and 2). Attachment of the ECG pads to the subjects' scales was simple and reliable, and removal post-recording was achieved either with gentle manual traction or the use of a proprietary spray designed for adhesive bandage removal (Eaze-Off™, Millpledge Veterinary). In both instances, removal was quick and atraumatic.

Due to the absence of limbs, positioning of the electrodes was based around a modified Einthoven triangle.^{13 14} The red electrode was placed on the right side and cranial to heart, the yellow on the left and cranial to heart, and the green on the left and caudal to heart (Figure 2). The ECG was recorded continuously for three minutes using both 25 and 50 mm sec⁻¹, at sensitivities of 10 and 20mV.

Standard ECG measurements were made from the lead 2 equivalent (negative electrode right and cranial to heart, positive electrode left and caudal to heart), with mean and standard deviations (SD) calculated from a representative run of three consecutive cardiac cycles, using paper speeds of 50 mm sec⁻¹ and sensitivities of 20mV. Heart rate was calculated from the number of R waves in a randomly selected 30-second period. The mean electrical axis (MEA) was extrapolated from the sum of the amplitudes of positive and negative deflections in leads 1 and 3 (Lead 1 defined as negative electrode is right and cranial to heart, positive electrode is left and cranial to heart. Lead 3 is defined as negative electrode left cranial to heart, positive electrode left and caudal to the heart).²⁶

Study-specific measurement points were established to facilitate interval and amplitude calculations. An isoelectric baseline was selected at the segment immediately following the T wave to address the persistent deviation from horizontal at the PQ and ST segments (Figure 3). As no clear Q or S waves were identifiable on any of the tracings, the QRS was referred to as an R wave, and the QT interval was measured from the start of the R wave to the end of the T wave and referred to as the RT interval (figure 3).

Data are expressed as mean \pm SD; statistical analysis was performed using commercial software (Minitab 17, Ltd, Coventry UK). Data were tested for normality (Anderson-Darling test), and correlations between length or bodyweight and heart rate or MEA were determined by Pearson's (normal) or Spearman's rho (not normal) tests. Differences between genders and bodyweight and length were determined by a two-sample *t*-test. For all tests, a *P* value < 0.05 was considered significant.

Results

The process of cardiac identification using ultrasound probe and coupling gel was simple, accurate and rapid. Subject acceptance of handling, scanning and placement of self-adhesive ECG pads was typically excellent, with the exception of a sub-set of individuals who required additional time to settle before commencing the ECG. High-quality ECG tracings were obtained in all subjects.

A sinus rhythm was recorded in all of the snakes, with R wave preceded by a P wave and followed by a T wave. P wave morphology varied slightly between leads, but was typically low in amplitude, short in duration and monophasic. On lead 2, 15/29 (52%) study subjects had negative P waves, while in 2/29 (7%) bi-phasic P waveforms were present (figure 4). Q and S waves were not identified on any lead on any of the tracings, therefore ventricular depolarisation consisted universally of

monophasic R waves, with a high amplitude, short duration and positive deflection on lead 2 (figure 4). T waves were highly variable between individuals, with 20/29 (69%) snakes exhibiting a negative waveform on lead 2. Otherwise, T waves were monophasic and commonly of moderate to low amplitude (figure 3).

There was a gradual downward sloping of the PR segment in 24/29 (83%) snakes, while the RT segment followed an upward slope in 20/29 (69%) snakes, making it difficult to establish the starting point of the T wave. PR and RT intervals were proportionally long, while TP segments were short. 11/29 (38%) snakes in this study demonstrated either pronounced shortening of the TP segment or early merging of the T and P waves (figure 5).

On leads other than lead 2, P, R and T waves could be identified on all tracings, with the exception of 5 subjects for whom T waves could not be identified on lead 1, 2 of which also had no identifiable P waves on lead 1. SV waves were not identifiable on any of the tracings.

Lead 2 ECG amplitude and interval duration values are shown in Table 1.

Parameter	Amplitude (mV)	Duration (sec)
P wave	0.107 +/- 0.045	0.064 +/- 0.016
T wave	0.180 +/- 0.127	N/A
R wave	1.061 +/- 0.395	0.107 +/- 0.019
PR interval	N/A	0.259 +/- 0.059
RT interval	N/A	0.585 +/- 0.145

Table 1. Lead 2 waveform in terms of wave amplitude (mV) and interval duration (sec), expressed as the mean +/- standard deviation (SD).

Statistics

There was no difference in bodyweight or length measurements between males and females (P = 0.634 and P = 0.506, respectively. Figure 6 (A and B)). Heart rate was always between 20 and 97 bpm and was not found to be significantly different between genders (P = 0.094. Figure 6 (D)) or correlated with weight or length (P =

0.972 and 0.396, respectively. Figure 7). MEA was always positive and displaced to the left, and was neither correlated with bodyweight nor length ($P = 0.910$ and $P = 0.771$, respectively. Figure 8), or influenced by gender ($P = 0.233$, T-value = 1.24, DF = 15. Figure 6 (C)).

The mean and SD for weight, length, heart rate (HR) and MEA, by group, are presented in table 2.

Parameter	Group (n=29) mean and SD	Female (n=10) Mean +/- SD	Male (n=19) Mean +/- SD
Weight (g)	609 +/- 216	583 +/-189	622 +/- 233
Length (cm)	114 +/- 24	117 +/- 25	113 +/- 24
Heart rate (bpm)	60 +/- 18	53 +/- 17	65 +/- 18
MEA (degrees)	66 +/- 12	64 +/- 13	70 +/- 11

Table 2. Weight, length, HR and MEA expressed as mean +/- SD values for group and gender (male and female)

Discussion

To the authors' knowledge, this is the first comprehensive descriptive study of ECGs from fully conscious and unrestrained corn snakes. We used a modified Einthoven's triangle that was atraumatic and well tolerated to generate high quality recordings that were suitable for interpretation.

Despite the absence of a specialised electrical conduction network, ECGs from all corn snakes in this study consisted of sequential atrial and ventricular depolarisations analogous to the P wave, PQ interval, QRS complex and T waves observed in mammals. In contrast to mammals, however, and as has been previously reported in reptiles,^{13 14} none of the ECGs had recognisable Q or S waves at standard sensitivities, suggesting that PR and RT intervals rather than PQ and QT intervals is more appropriate terminology in this species.

We identified several key features of the PRT sequence that are relevant to future clinical and research use in this and potentially other snake species.

First, P waves have heterogeneous polarity between individuals and can be positive, negative or, biphasic (figure 3). It is also noteworthy that P waves were not preceded by sinus venosus waves, which have been variably identified in other reptile studies.^{19 22 27} Cardiac mass in reptiles is disproportionately small when compared to mammals or birds, representing only 0.2% to 0.3% of body mass.¹⁸ We speculate that action potentials (AP) from this myocardial structure may be masked by preceding T waves and/or background APs from skeletal muscle contraction.^{14 24 27}

Second, although an AV node has not been identified in snakes, the PR interval, and hence atrioventricular conduction, is prolonged in a similar way to that mediated by the AV node in mammals. It has been suggested that a long PR interval in reptiles could reflect slow AP conduction along tortuous pathways through the AV canal, slow rates of cardiac myocyte depolarisation, or an absence of fast-propagating gap junctions from the AV canal.²⁸ None of these mechanisms have been investigated in the corn snake but do represent possible areas for future research. The PQ interval in mammals is under autonomic control, and small changes in such a long PR interval in snakes could represent an easily measurable marker of autonomic status and metabolic health.

Third, R waves were the tallest complexes of the sequence and always positive on lead 2, findings which suggest homology to mammals by demonstrating a rapid systolic phase and action potential (AP) propagation towards lead 2, respectively. This feature also provides an easily identifiable waveform when interpreting corn snake ECG tracings (figure 3).

Fourth and finally, the T wave was commonly found to be very wide and of variable morphology between corn snakes, as has been reported in other reptile species.^{21 27}

²⁹ This segment was approximately 2-3 times longer than the QT of mammals, but,

similar to mammals, was around twice the duration of the PR (PQ) interval. High numbers of snakes demonstrated PR depression followed by a curvilinear elevation from the end of the R (J-point) to the T wave, which caused difficulties in establishing the isoelectric baseline along traditional mammalian lines. This prompted the creation of a new baseline at the early part of the TP segment (figure 4) which was consistently more level. In human cardiology, PQ elevation and ST depression, either separately or concurrently, are clinically significant and associated with myocardial infarction and myocarditis.^{30 31} Given the high number of subjects in our study demonstrating this feature and the general good health of the cohort, these segmental fluctuations are unlikely to have the same pathological importance in corn snakes, although we could speculate that PR elevation and ST depression indicate a normal degree of myocardial ischaemia in the snake heart. The embryonic hearts of birds and mammals demonstrate a great inherent tolerance to ischemia³² and as the hearts of adult reptiles are structurally very similar it is possible that this is a shared characteristic. An alternative explanation could be mechanical and related to the relatively unique anatomy of the cardiac region in snakes. The attachments of the ophidian heart in the cranial coelom are loose when compared to their mammalian counterparts and allow for the passage of whole prey items. They could permit a rhythmic swinging of the heart through the contraction cycle, leading to a regular oscillation of the ECG baseline. This could be investigated further by recording an ECG, using the arrangement of ECG pads herein described, while simultaneously performing echocardiography.

The heart rates we obtained are in general agreement with those reported in other ophidian studies and are not dissimilar from other representatives of the class reptilia (Tables 3 and 4).

Study	Heart rate range (bpm)
Clark and Marx, 1960 ¹⁸	9 -72
Mullen, 1967 ¹⁹	22 – 136
Valentinuzzi et al. 1969 ²⁰	13 – 62
Anderson et al. 1999 ³³	57 - 73
Lewis et al, unpublished data	42 - 78

Table 3. Snake heart rate ranges by study.

Study	Species	Heart rate range (bpm)
Heaton-Jones and King, 1994 ³⁴	American alligator	33 – 42
Holtz and Holtz, 1995 ³⁵	Red-eared slider	16 – 36
Martinez-Silvestre et al. 2003 ³⁶	Gomaran giant lizard	35 – 60
Tan et al. 2013 ³⁷	Marbled water monitor	26 - 58
Hunt, 2015 ²⁷	Central bearded dragon	24 – 170

Table 4. Reptile heart rates – study, species and range.

They are however much slower than mammals and reflect the approximately 10-fold lower metabolic rate seen in reptiles.^{13 28} The heart rate of snakes can be affected by a wide range of intrinsic and extrinsic factors, but in general it is increased with elevated body temperature, small body size, external stimuli (such as handling), recent meal ingestion and gravidity.^{16 24 38-41} Previous studies involving ECG in snakes have been highly variable in design and have frequently included data from small cohort numbers, or large numbers of species restrained by different physical and chemical methods.¹⁸⁻²² Our data, obtained from only one species, came from a larger study cohort and our protocol minimised confounders. Nonetheless, our data resemble closely those obtained where confounding factors have not been so rigorously controlled,^{19 42} suggesting that they may have less influence on heart rate and cardiac conductivity than previously supposed.

Typically in mammals, as heart rate increases the period of time between the T wave and the P wave of the next cardiac cycle shortens.²⁶ In the study here reported, shortening of the TP interval was also observed in snakes with heart rates at the upper end of the population range. In some cases this shortening was so extreme that there was merging of T and P waves. Germer et al. (2015)⁴³ mention T and P wave merging in a group of geckos, while Mullen (1967)¹⁹ and Jacob and McDonald (1975)²⁹ describe wave overlap at the TP interval or “masking” of the P wave by the T wave in cohort sub-groups of wild caught snakes. These authors do not speculate on the possible causes of this finding, but Clarke and Marx (1960)¹⁸ identify handling stress as an important cause of increased heart rates in wild caught snakes. In our study, while all possible efforts were made to reduce stress at the time of ECG measurement and although all individuals were captive bred pet animals, it was clear that some snakes were initially wary of interference and took greater time to settle. This group of snakes tended to have higher heart rates and more pronounced TP shortening. We conclude that corn snakes have an inherent variability to handling tolerance but also, and more importantly, that marked shortening of the TP segment or TP merging may be an additional useful, quantifiable indicator of stress not only for captive corn snakes but for snakes in general, a collection of animals for which behavioural indicators of stress can be very vague. The physiological advantage of this response is not clear. Atrial depolarisation so close to ventricular repolarisation in snakes could significantly decrease time available for passive ventricular filling (which predominates in mammals), even at heart rates considered low in mammals, increasing reliance on the atrial contribution to ventricular diastole. If confirmed by, for example spectral and tissue Doppler echocardiography, it would suggest a profound difference in the

mechanics of diastole in corn snakes compared to those in turtles and pythons, for whom ventricular suction is thought to play an important role.²⁸

We also investigated whether gender might have an influence on HR.

Follicular genesis can occur in female snakes independent of the presence males and sporadically throughout a captive snake's life. It would be reasonable to expect that the elevated metabolic rate associated with this condition would lead to an increase in HR. We did not attempt to determine the level of ovarian activity in individual females through an additional ultrasound examination due to concern of increased confounding stress effects. However, we did not identify a significant increase in HR in our female corn snake population when compared to males. Other factors such as weight and snout-to-vent length were also found to have no significant relationship with HR. Therefore, we believe that the range of HR we recorded in both male and female corn snakes are representative of the general corn snake population.

The mean electrical axis (MEA) in reptiles has historically been regarded as very challenging (if not impossible) to determine and of limited clinical value.¹⁴ By contrast, in the study here reported identification and measurement of R waves on leads 1 and 3 was readily performed and the MEA was easily calculated. Importantly, the technique we describe produces a MEA through electrode placement and draws similarities to companion mammals, rather than MEA as defined by cardiac alignment and physical measurements. Yielded values demonstrated a left axis dominance (table 2), indicating that it is the left side of the common ventricle in corn snakes that makes up most of the myocardium. This axial dominance is similar to mammals, where the left ventricle is larger than the right, but is also similar to the few other reptile species for which a MEA has been calculated

26 36 34, indicating a common asymmetrical distribution of ventricular muscle mass in reptiles. We also found that MEA values produced in this study were highly conserved between individuals, suggesting a predictable direction of electrical flow through the myocardium and therefore a broadly uniform positioning of the heart within the coelom. We suggest that the ECG in corn snakes generates a vector of a MEA that is quantifiable and has the potential to aid diagnosis of cardiac disease in snakes, similar to its use in mammals. Further, that the technique described herein will permit the calculation of MEA to the benefit of future ophidian studies.

Limitations

There were a number of limitations to this study. First, the exact ages of the snakes involved was not always known. Size (in terms of snout-to-vent length and weight), which was used to classify individuals as adult, may be inaccurate as growth can be influenced by a number of factors including gender, long-term nutrition and captive environment. However, known adult animals were present in our study population and no difference in body weight or length measurements was identified between male and female snakes. It is therefore reasonable to consider the cohort as homogeneous in terms of size and, by extension, adult age. Second, despite a comprehensive clinical examination, the absence of cardiac or non-cardiac disease could not be excluded from our study group. Inclusion of routine blood sampling and comprehensive echocardiography would have helped to identify systemic disease and structural or functional cardiac disease, respectively. These investigations, however, fell outside the scope of the study hypothesis and may have introduced a significant confounding stress effect on our data. Furthermore, the limits of ethical approval precluded invasive procedures (such as blood sampling), or undue stress

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through prolonged or repeated handling. Third, the cohort size was insufficient to provide reference ranges for this species. However, to the authors' knowledge, this is the largest single-species ophidian study that has been published. Finally, there were twice as many males as females in this study which could have introduced a gender bias that was not identifiable on the variables we measured. However, excluding females from analysis would have reduced the statistical power of the study.

Conclusion

As hypothesised, ECG waveforms in corn snakes are morphologically recognisable and can be characterised and interpreted in a similar way to ECGs obtained from mammals. However, there are important differences such as heart rate and, particularly, TP intervals that may be useful in identifying corn snakes experiencing stress. The data we present could help provide reference ranges for ECG parameters in this species, aiding the clinical application of ECGs in general and specialist practice.

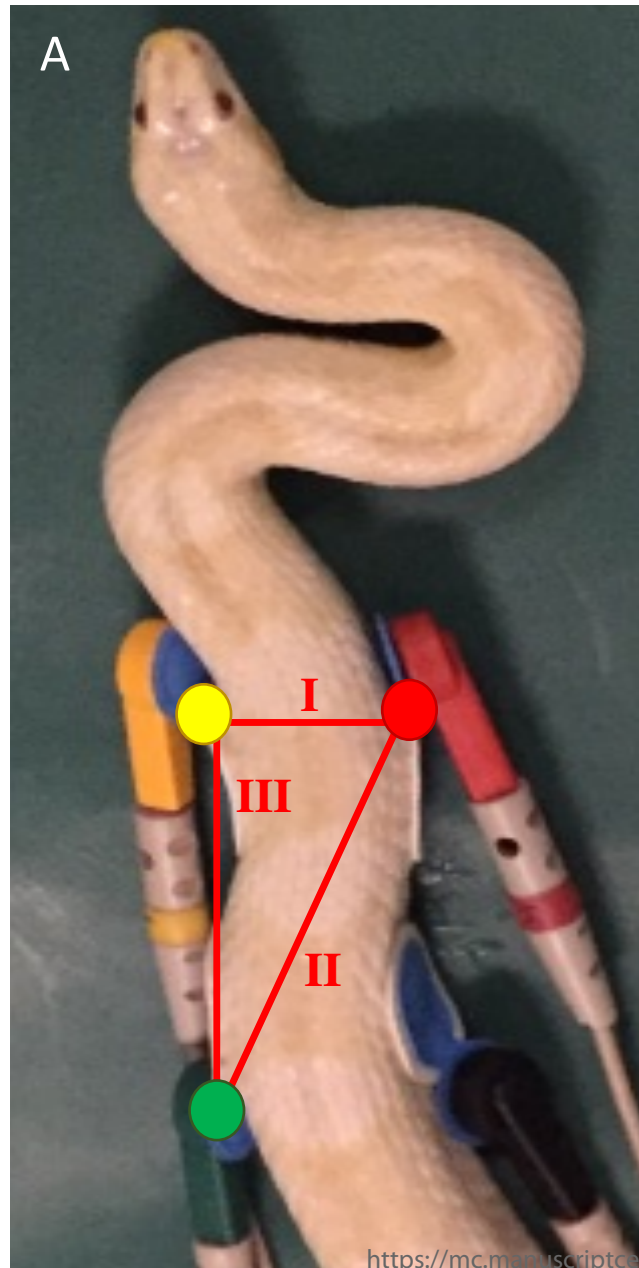
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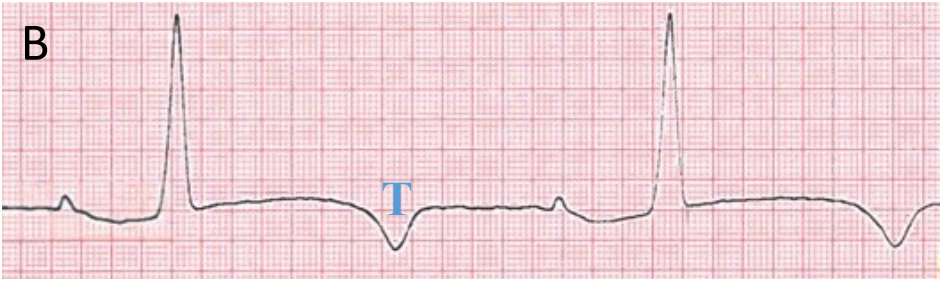
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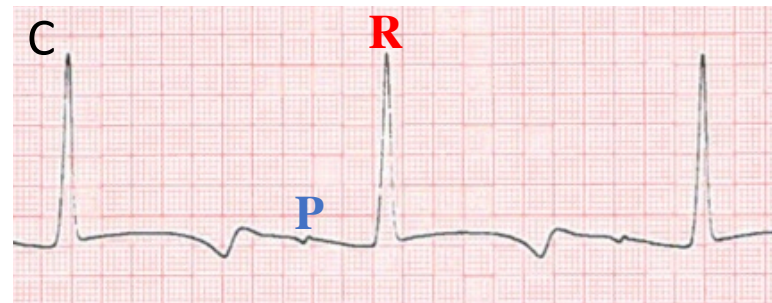
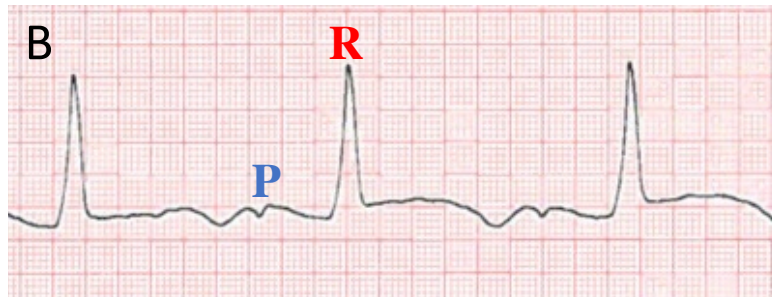
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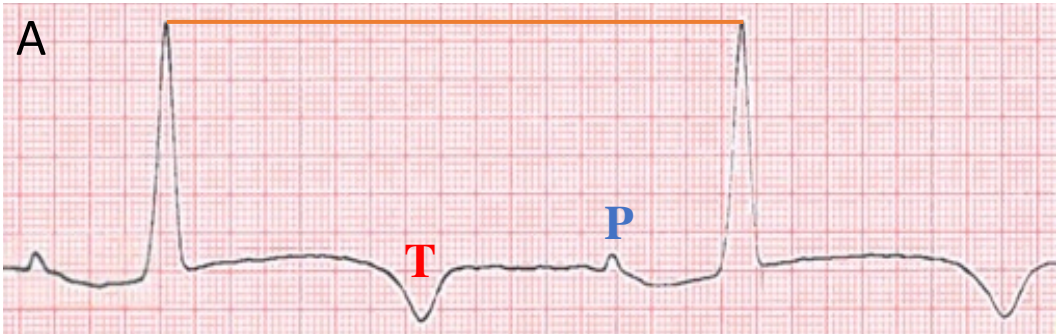








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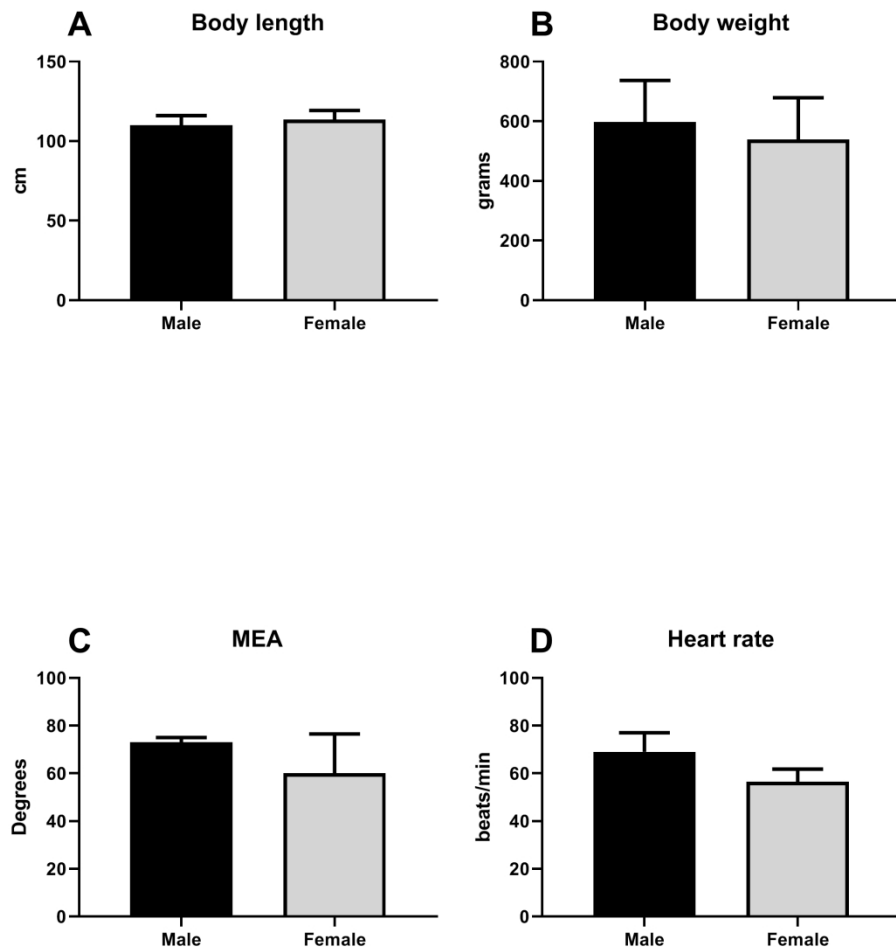


Figure 6. Bar charts comparing male (black bars) and female (grey bars) corn snakes in terms of mean body length (A), body weight (B), MEA (C) and HR (D).

208x210mm (300 x 300 DPI)

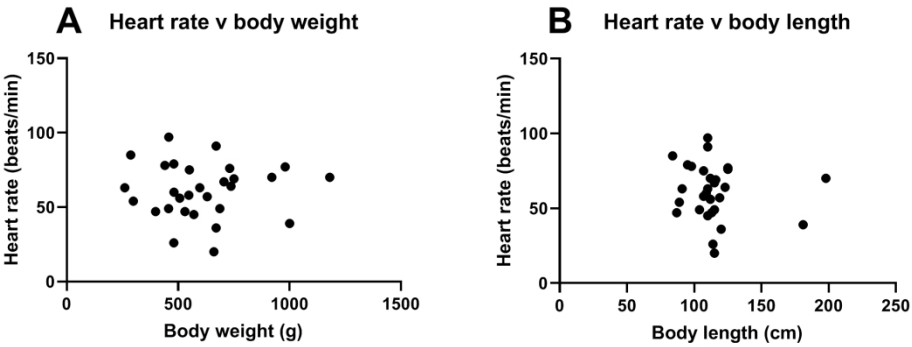


Figure 7. Scatter plots for heart rate against body weight (A), and body length (B), indicating no correlation between variables.

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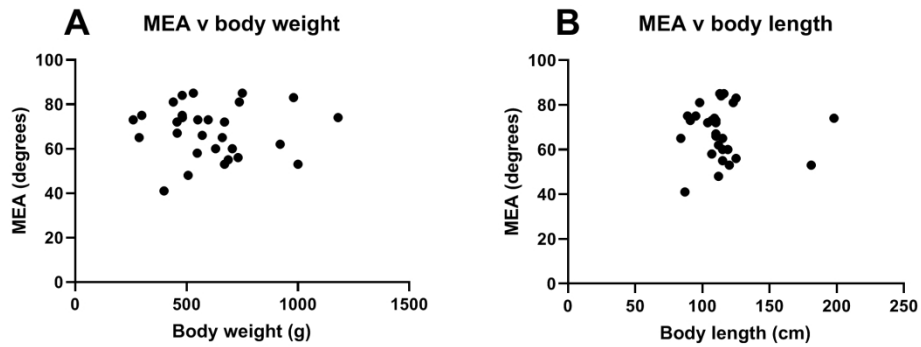


Figure 8. Scatter plots for MEA against body weight (A), and body length (B), indicating no correlation between variables.

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